

VISIT...

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WET-COLUMN CHROMATOGRAPHY

Wet-column chromatography, as you may have guessed, is chromatography carried out on a **column of adsorbent**, rather than a layer. Not only is it cheap, easy, and carried out at room temperature but also you can separate large amounts, *gram quantities*, of mixtures.

In column chromatography, the adsorbent is either alumina or silica gel. Alumina tends to be basic and silica gel tends to be acidic. If you try out an eluent (solvent) on silica gel plates, you should use a silica gel adsorbent. And if you have good results on alumina TLC, use an alumina column.

Now you have a *glass tube as the support* holding the adsorbent in place. You dissolve your mixture and put it on the adsorbent at the top of the column. Then you wash the mixture down the column using at least one eluent (solvent), perhaps more. The compounds carried along by the solvent are washed *entirely out of the column*, into separate flasks. Then you isolate the separate fractions.

PREPARING THE COLUMN

1. The adsorbent is supported by a glass tube with either a stopcock or a piece of tubing and a screw clamp to control the flow of eluent (Fig. 129). You can use an ordinary buret. What you will use will depend on your own lab program. Right above this control you put a wad of cotton or glass wool to keep everything from falling out. *Do not use too much cotton or glass wool, and do not pack it too tightly.* If you ram the wool into the tube, the flow of eluent will be very slow, and you'll be in lab until next Christmas waiting for the eluent. If you pack it *too loosely*, all the stuff in the column *will fall out*.
2. At this point, fill the column half-full with the least polar eluent you will use. If this is not given, you can guess it from a quick check of separation of the mixture on a TLC plate. This would be the advantage of an alumina TLC plate.
3. Slowly put sand into the column through a funnel until there is a 1 cm layer of sand over the cotton. Adsorbent alumina or silica gel is so fine that either is likely to go through cotton but not through a layer of fine sand.
4. During this entire procedure, *keep the level of the solvent above that of any solid material in the column!*
5. Now slowly add the adsorbent. Alumina or silica gel are adsorbents and they'll suck up the solvent. When they do, heat is liberated. The solvent may boil and *ruin the column*. Add the adsorbent slowly! Use

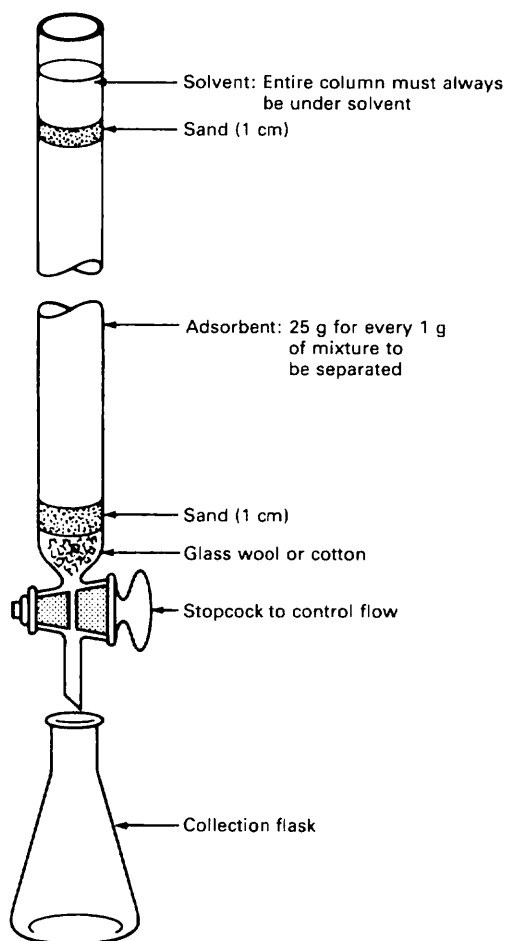


Fig. 129 Wet-column chromatography setup.

about 25 g of adsorbent for every 1 g of mixture you want to separate. While adding the adsorbent, *tap or gently swirl the column to dislodge any adsorbent or sand on the sides*. You know, a plastic wash bottle with eluent in it can wash the stuff down the sides of the column very easily.

6. When the alumina or silica gel settles, you normally have to add sand (about 1 cm) to the top to keep the adsorbent from moving around.
7. Open the stopcock or clamp and let solvent out until the level of the solvent is just above the upper level of sand.
8. *Check the column! If there are air bubbles or cracks in the column, dismantle the whole business and start over!*

COMPOUNDS ON THE COLUMN

If you've gotten this far, congratulations! Now you have to get your mixture, the analyte, on the column. Dissolve your mixture in the same solvent you are going to put through the column. Try to keep the volume of the solution of mixture as *small as possible*. If your mixture does not dissolve entirely (*and it is important that it do so*), check with your instructor! You might be able to use different solvents for the analyte and for the column, but this isn't as good. You might use the *least polar* solvent that will dissolve your compound.

If you must use the column eluent as the solvent, and not all the compound will dissolve, you can filter the mixture through filter paper. Try to keep the volume of solution down to 10 ml or so. After this, the sample becomes unmanageable.

1. Use a pipet and rubber bulb to slowly and carefully add it to the top of the column (Fig. 130). *Do not disturb the sand!*
2. Open the stopcock or clamp and let solvent flow out until the level of the solution of compound is slightly above the sand. *At no time let the solvent level get below the top layer of sand!* The compound is now "on the column."

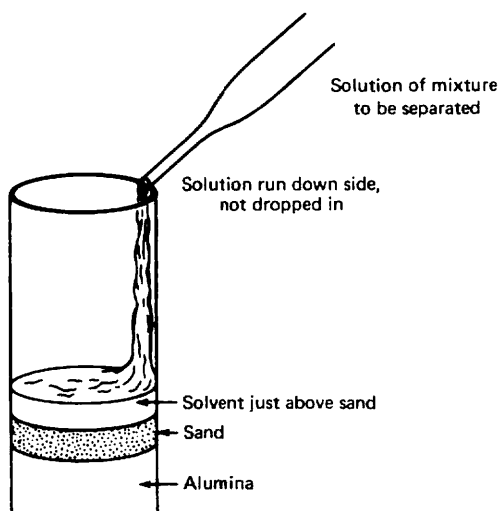


Fig 130 Putting compounds on the column by pipet.

3. Now add eluent (solvent) to the column above the sand. *Do not disturb the sand!* Open the stopcock or clamp. Slowly let eluent run through the column until the first compound comes out. Collect the different products in Erlenmeyer flasks. You may need lots and lots of Erlenmeyer flasks. *At no time let the level of the solvent get below the top of the sand!* If necessary, stop the flow, add more eluent, and start the flow again.

VISUALIZATION AND COLLECTION

If the compounds are colored, you can watch them travel down the column and separate. If one or all are colorless, you have problems. So:

1. *Occasionally* let one or two drops of eluent fall on a clean glass microscope slide. Evaporate the solvent and see if there is any sign of your crystalline compound! This is an excellent spot test, but don't be confused by nasty plasticizers from the tubing trying to put one over on you, pretending to be your product.
2. Put the narrow end of a "TLC spotter" to a drop coming off at the column. The drop will rise up into the tube. Using this loaded spotter, *spot, develop, and visualize a TLC plate with it*. Not only is this more sensitive, but also you can see whether the stuff coming out of the column is pure (see Chapter 26, "Thin-Layer Chromatography"). You'll probably have to collect more than one drop on a TLC plate. If it is very dilute, the plate will show nothing, even if there actually is compound there. It is best to sample four or five consecutive drops.

Once the first compound or compounds have come out of the column, those that are left may move down the column much too slowly for practical purposes. Normally you start with a nonpolar solvent. But by the time all the compounds have come off, it may be time to pick up your degree. The solvent may be too nonpolar to kick out later fractions. So you have to decide to change to a more polar solvent. This will kick the compound right out of the column.

To change solvent in the middle of a run:

1. Let the old solvent level run down to just above the top of the sand.
2. *Slowly add new, **more polar solvent** and do not disturb the sand.*

You, and you alone, have to decide if and when to change to a more polar solvent. (Happily, sometimes you'll be told.)

If you have only two components, start with a nonpolar solvent, and when you are sure the first component is completely off the column, change to a really polar one. With only two components, it doesn't matter what polarity solvent you use to get the second compound off the column.

Sometimes the solvent evaporates quickly and leaves behind a "fuzz" of crystals around the tip (Fig. 131). Just use some fresh solvent to wash them down into the collection flask.

Now all the components are off the column and in different flasks. Evaporate the solvents (*No flames!*), and lo! The crystalline material is left.

Dismantle the column. Clean up. Go home.

WET-COLUMN CHROMATOGRAPHY: MICROSCALE

Occasionally, you'll be asked to clean and/or dry your product using **column chromatography** by pipet. It's a lot like large-scale wet-column chromatography.

One problem with this pipet column is that there's no stopcock. Once it starts running, it goes until it runs out. And you can't let the column dry out either. So it looks like you're committed to running the column once you prepare it.

In general (Fig. 132):

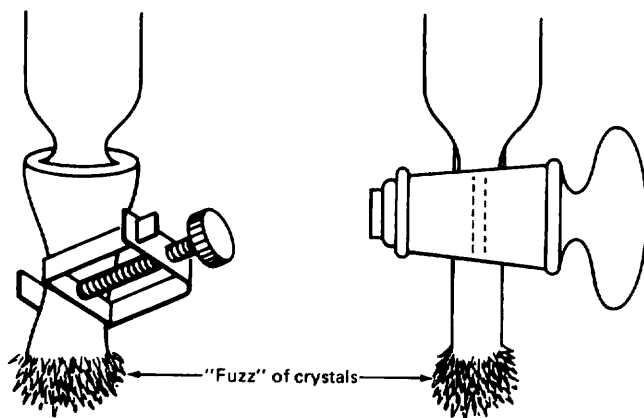


Fig. 131 A growth of crystals occurs as the eluent evaporates.

1. Shorten the tip of a Pasteur pipet (see “Pipet Cutting” in Chapter 7, “Pipet Tips”) and tamp cotton into the tip as usual.
2. Add about 50 mg of fine sand.
3. Now comes the adsorbent, about 500 mg or so. Gently tap the pipet to pack this down.
4. Put a bit more sand on top to keep the adsorbent from flying around.
5. Now slowly add the solvent and let it wet the entire column.
6. Dissolve your sample in a minimum amount of solvent—the least polar solvent it’ll dissolve in—and add this solution, slowly, to the top of the pipet. Don’t fill the pipet with liquid; drip the solution onto the sand.
7. With the sample all on the column, just below the sand, add a small column of **elution solvent**. This may or may not be different from the solvent you dissolved your sample in.
8. Wait and watch. Watch and wait. Do not ever let the column run dry. Collect the drops off the end in a series of fractions in some small containers.

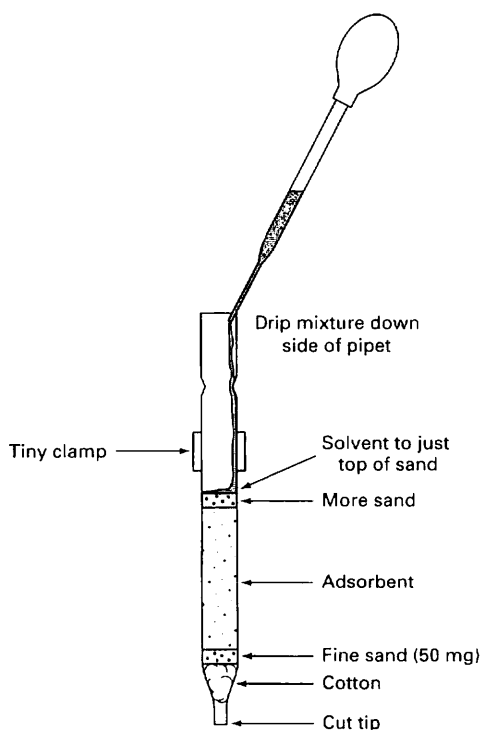


Fig. 132 Pasteur pipet wet-column chromatography.